

Copy Number Variation analysis in adults with catatonia confirms haploinsufficiency of *SHANK3* as a predisposing factor

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ABSTRACT

Background: Catatonia is a motor dysregulation syndrome co-occurring with a variety of psychiatric and medical disorders. Response to treatment with benzodiazepines and electroconvulsive therapy suggests a neurobiological background. The genetic etiology however remains largely unexplored. Copy Number Variants (CNV), known to predispose to neurodevelopmental disorders, may play a role in the etiology of catatonia.

Methods: This study is exploring the genetic field of catatonia through CNV analysis in a cohort of psychiatric patients featuring intellectual disability and catatonia. Fifteen adults admitted to a psychiatric inpatient unit and diagnosed with catatonia were selected for array Comparative Genomic Hybridization analysis at 200kb resolution. We introduced a CNV interpretation algorithm to define detected CNVs as benign, unclassified, likely pathogenic or causal with regard to catatonia.

Results: Co-morbid psychiatric diagnoses in these patients were autism, psychotic or mood disorders. Eight patients were found to carry rare CNVs, which could not be classified as benign, comprising 6 duplications and 2 deletions. Microdeletions on 22q13.3, considered causal for catatonia, were detected in 2 patients. Duplications on 16p11.2 and 22q11.2 were previously implicated in psychiatric disorders, but not in catatonia, and were therefore considered likely pathogenic. Driven by the identification of a rare 14q11.2 duplication in one catatonic patient, additional patients with overlapping duplications were gathered to delineate a novel susceptibility locus for intellectual disability and psychiatric disorders on 14q11.2, harboring the gene *SUPT16H*. Three remaining variants respectively on 2q36.1, 16p13.13 and 17p13.3 were considered variants of unknown significance.

Conclusion: The identification of catatonia-related copy number changes in this study, underscores the importance of genetic research in patients with catatonia. We confirmed that 22q13.3 deletions,

affecting the gene *SHANK3*, predispose to catatonia, and we uncover 14q11.2 duplications as a novel susceptibility factor for intellectual and psychiatric disorders.

Key words: catatonia; psychiatric disorder; *SHANK3*; 14q11.2 duplication; *SUPT16H*

INTRODUCTION

Catatonia is a motor dysregulation syndrome characterized by alternation of excessive and reduced mobility, often in association with speech abnormalities. It should be considered in any patient with marked deterioration in psychomotor function and overall responsiveness (movement, speech, self-care, skills). Prevalence estimates of catatonia among psychiatric patients range from 7.6 to 38% (Fink, 2009; Taylor and Fink, 2003). Although common and severe, catatonia remains poorly recognized. There have been efforts to enhance early diagnosis, as reflected by changes in catatonia classification in DSM-5, where it appears as a distinct clinical-diagnostic entity (Tandon et al., 2013). A clinical diagnosis of catatonia is based on the presence of at least 3 of the following DSM-5 criteria: catalepsy, waxy flexibility, stupor, agitation, mutism, negativism, posturing, mannerisms, stereotypy, grimacing, echolalia or echopraxia. Symptoms may present in different combinations and co-occur with a variety of other psychiatric or medical conditions (Daniels, 2009). This clinical heterogeneity hampers early recognition and treatment, which are important as catatonia is sometimes associated with autonomic symptoms, that can be life threatening.

Regardless of the comorbid pathology, there is a unique response to treatment with benzodiazepines, zolpidem and electroconvulsive therapy (ECT), suggesting a biological etiology separate from other psychiatric disorders (Dhossche et al., 2010; Javelot et al., 2015). Benzodiazepines and ECT are agonists of the inhibitory function of the GABA-A receptor complex. Their therapeutic effect on catatonia suggests a dysfunctional neurotransmission as the underlying neurobiological mechanism. A possible genetic etiology underlying disrupted synaptic transmission in the development of catatonia, is supported by the

occurrence of catatonic symptoms in patients with Prader-Willi Syndrome (PWS) (Dhossche and Bouman, 1997; Poser and Trutia, 2015). PWS is caused by the lack of expression of genes on the paternally derived 15q11q13 chromosomal segment, including a set of genes coding for particular subunits of the GABA_A receptor, namely *GABRB3*, *GABRA5* and *GABRG3*, which encode the $\beta 3$, $\alpha 5$, and $\gamma 3$ subunits, respectively (Coghlan et al., 2012). This has led to the assumption that altered inhibitory GABAergic neurotransmission may underlay catatonia in PWS (Dhossche et al., 2005). In addition to this GABAergic hypothesis, excess glutamate or hyperactivity of glutamate receptors such as the N-Methyl-D-Aspartate receptor (NMDAR), seem to be involved as well, as catatonic symptoms in selected cases resolve by treatment with the NMDA antagonist Amantadine (Northoff et al., 1999). The beneficial effects of NMDA antagonists on catatonia are ascribed to recovery of GABA-A function in brain regions, that were previously GABA-A deficient due to NMDA hyperactivity (Carroll et al., 2007).

Despite the evidence of a role of disturbed neurotransmission involving different synaptic receptors, studies on the genetic etiology of catatonia are scarce. Three studies reported familial transmission with anticipation and parent-of-origin effect in periodic catatonia (Beckmann et al., 1996; Stober et al., 1995; Stober et al., 1998). The same research group reported linkage of periodic catatonia to 15q15 and 22q13 (Stober et al., 2000). Whereas the causative gene for catatonia on 15q15 remains obscure, catatonia related to 22q13 has been attributed to loss of one functional copy of the *SHANK3* gene, as loss-of-function mutations and deletions affecting this gene were found in catatonic patients with intellectual disability and comorbid psychiatric disease (Serret et al., 2015). In addition, catatonia has been recurrently found in association with Down syndrome (Ghaziuddin et al., 2015) and the 22q11 deletion syndrome (Faedda et al., 2015).

More recent genomic technologies such as chromosomal microarray and massive parallel sequencing may help to identify other chromosomal regions and genes playing a role in the etiology of this motor

dysregulation disorder. In this study, we performed copy number analysis in 15 adults with intellectual disability and catatonia to identify chromosomal deletions and duplications predisposing to this psychiatric disorder.

MATERIALS AND METHODS

Patients

From January 2005 until January 2015, 283 adult psychiatric patients with intellectual disability were recruited from an inpatient unit in the St-Camillus Psychiatric Hospital in Bierbeek, Belgium, for clinical and psychiatric evaluation. During psychiatric hospitalization, 15 out of these 283 adults were clinically diagnosed with catatonia. Diagnosis was made by a psychiatrist and an educational psychologist with expertise in intellectual disability. Medical files were analyzed retrospectively to collect data on their medical history, cognitive functioning and psychiatric comorbid diagnosis, and to score catatonia based on DSM-5 criteria. Because of the unique response of catatonia to treatment with benzodiazepines, such as lorazepam, benzodiazepine-like products such as zolpidem, and/or electroconvulsive therapy, response to such treatment was added as an additional criterion, favoring the diagnosis of catatonia. For all patients, a genome-wide screen for copy number alterations was performed by array comparative genomic hybridization.

The study was approved by the ethical committee at the University Hospitals Leuven. Appropriate informed consent was obtained from all participating patients or their legal representatives.

Array Comparative Genomic Hybridization (array CGH)

Genomic DNA was extracted from peripheral leukocytes of EDTA-treated blood according to standard procedure guidelines. Array CGH was carried out using the Oxford Gene Technology (OGT) CytoSure™ ISCA oligoarray set (Oxford Gene Technology, Oxford, UK) containing 180k DNA oligonucleotides with a

minimum resolution of 200kb. Microarray hybridization and copy number variant (CNV) analysis were performed according the manufacturer's instructions. All genome coordinates were according to NCBI human genome build 37 (hg19, February 2009).

Copy Number Variant (CNV) interpretation algorithm

We introduced an algorithm to classify detected CNVs as benign, unclassified, likely pathogenic or causal for catatonia (supplementary figure 1). (1) A CNV was considered causal, either when this CNV was completely overlapping with chromosomal syndromes known to cause catatonia, or when this CNV was harboring catatonia-related genes known to be dosage sensitive. (2) CNVs were considered likely pathogenic, when the CNV affects genes or the critical region of a chromosomal syndrome known to be implicated in intellectual disability or psychiatric disease, but not in catatonia. A list of known chromosomal syndromes and genes, related to intellectual disability and psychiatric disease is based on an internal database, and is available upon request. (3) CNVs affecting regions smaller than 200kb and not fulfilling the criteria under (1) or (2) were discarded from further analysis. (4) CNVs known to be prevalent in more than 1% of the normal population (based on a local database with over 7000 individuals), were considered benign. (5) All remaining rare CNVs were considered variants of unknown significance, and were further evaluated, based on gene content (OMIM, literature), inheritance and phenotype correlations with similar chromosomal variants, reported in literature or in publicly available databases, such as DECIPHER or the database of genomic variants. Parents were tested when available to determine the inheritance of causal chromosomal imbalances and rare CNVs of unknown significance.

RESULTS

Fifteen patients (12 males, 3 females) clinically diagnosed with catatonia were selected among 283 intellectually disabled psychiatric adults from a single inpatient psychiatric unit. Array CGH results and clinical descriptions, including response to treatment, are presented in table 1, table 2 and supplementary table 1. Intellectual disability ranged from mild to severe. The age of the patients ranged from 29 to 63 years. Ten patients responded to lorazepam, zolpidem or ECT. Three adults with predominant psychotic symptoms (patient 6, 12 and 13) were treated with clozapine, which is known to be incompatible with concomitant use of lorazepam. Two patients (patients 9 and 14) did not receive any of these treatments.

No clinical genetic diagnosis could be established in any of these patients, except for patient 3, who was diagnosed with Down syndrome, confirmed by fluorescence *in situ* hybridization. Array CGH analysis in this male was performed, to ensure that no additional CNVs, causing or aggravating catatonia, were present. CNVs were classified in accordance with our CNV interpretation algorithm discussed above. Seven patients (47%) were found to carry only benign CNVs (supplementary table 1). In the remaining 8 patients (53%), one trisomy 21 and 8 rare submicroscopic imbalances, which could not be classified as benign, were detected. Three of these variants were established predisposing factors for catatonia: 22q13.3 deletions including the *SHANK3* gene in patients 1 and 2, and trisomy 21 in patient 3 were considered causal (Ghaziuddin et al., 2015; Serret et al., 2015). In patients 4 and 5, duplications on 16p11.2 and 22q11.2 were identified. These are known susceptibility loci for psychiatric pathologies and intellectual disability (Fernandez et al., 2010; Lo-Castro et al., 2009; Shinawi et al., 2010; Szatkiewicz et al., 2014; Wentzel et al., 2008), but they have not previously been linked to catatonia. We therefore considered them as likely pathogenic. The remaining 4 CNVs detected were rare duplications of unknown significance, affecting regions on 2q36.1, 14q11.2, 16p13.13 and 17p13.3. These duplications have hitherto not been described as predisposing factors for intellectual disability nor for psychiatric disorders,

and were subjected to a literature search. The 22q13.3 deletion in patient 1 occurred *de novo*. For the remaining patients, no parental samples to define inheritance were available.

Patient 3, who was clinically diagnosed with Down syndrome, carried a 268 kb duplication on 2q36.1 in addition to the trisomy 21. This region was not reported as polymorphic in our locally curated CNV database, nor in the Database of Genomic Variants. An overlapping duplication was detected in one DECIPHER patient (DECIPHER 287451), who presented psychiatric problems, motor delay and seizures, and who was found to carry 2 additional duplications of unknown significance on 19q13. None of the duplicated genes within this 2q36.1 region have been linked to psychiatric pathology previously. Therefore, it is currently unclear if this variant may have caused or aggravated the psychiatric pathology or catatonia in this patient. A similar observation was made for patient 7, who carries a 289kb duplication on 16p13.13p13.12. Although an identical duplication was found in our local database in a patient (DECIPHER 288002) with autism, learning disabilities and short stature, this region has not been linked to psychiatric or developmental disorders in any other patient. The duplication on 17p13.3, which was detected in patient 8, encompassed *SLC43A2*, *SCARF1*, *RILP* and *PRPF8*. Duplications on 17p13 affecting *YWHAE*, *CRK* and/or *LIS1* (*PAFAH1B1*), are known susceptibility factors for intellectual disability, hypotonia, autism, behavioral problems, brain malformations, facial dysmorphic features, and hand or feet anomalies (Bruno et al., 2010; Curry et al., 2013). The current duplication did however not affect these genes. Moreover, this duplication partially overlapped with copy number gains in the normal population (Coe et al., 2014; Cooper et al., 2011). Therefore, this variant was considered unclassified, rather than likely pathogenic.

Finally, patient 6 was found to carry a 288kb duplication on 14q11.2. This duplicated region (chr14: 21,567,757-21,856,165) has hitherto not been reported in normal controls in the Database of Genomic Variants, nor in our locally curated database. Excluding CNVs larger than 5Mb in size, thirteen additional

patients with overlapping duplications were retrieved from literature and DECIPHER. Parental analysis of the duplication could not be established for the index patient, neither for five additional patients with 14q11.2 duplications. The duplication occurred *de novo* in five and was maternally inherited in three patients. Clinical details were obtained from 8 patients, including three previously reported patients (D'Angelo et al., 2013; Smyk et al., 2016; Vuillaume et al., 2014) and 5 patients reported in DECIPHER (DECIPHER 289620, 289709, 277175, 27947 and 256272). A clinical and molecular overview is depicted in table 3 and figure 1. Supplementary table 2 features the remaining five DECIPHER patients, from whom limited (DECIPHER 284790, 287656 and 258497) or no clinical data (DECIPHER 322324 and 292187) could be retrieved. Excluding the latter two, all patients presented with either intellectual disability or behavioral/psychiatric disorders. Therefore, as per our CNV interpretation algorithm, this 14q11.2 duplication was considered causal for the delayed development, and likely pathogenic for the catatonia in patient 6.

DISCUSSION

Rare CNVs which could not be classified as benign according the CNV interpretation algorithm for catatonia, were detected in 8 out of 15 intellectually disabled patients with catatonia and comorbid psychiatric disease (53%). In 3 out of 15 patients (20%), the genetic etiology of catatonia was established: patients 1 and 2 were diagnosed with a 22q13.3 deletion, causing Phelan-McDermid syndrome, and patient 3 with trisomy 21.

SHANK3 has been identified as the critical gene in neurological and behavioral aspects of Phelan-McDermid syndrome (Phelan and McDermid, 2012). Loss of *SHANK3* function can be caused either by chromosomal loss on 22q13.3 or by smaller intragenic deletions or point mutations (Durand et al., 2007). Patients 1 and 2 both carry an identical 97kb deletion, partially affecting *SHANK3*. Similar deletions have been reported in DECIPHER patients with Phelan-McDermid syndrome. *SHANK3* is a scaffolding protein,

enriched in the postsynaptic glutamatergic excitatory synapses and interacting with various synaptic molecules. A major interaction partner of SHANK3 is the NMDAR complex (Duffney et al., 2013), well known for its role in anti-NMDAR-encephalitis. Interestingly, this non-infectious encephalitis has specific clinical features compatible with catatonia like catalepsy, stupor, mutism, posturing and stereotypical movements (Dhossche et al., 2011; Kramina et al., 2015). This suggests dysfunction of the postsynaptic NMDAR-SHANK3 unit in at least some of the catatonic patients. The NMDAR is widely expressed both in excitatory glutamatergic and inhibitory GABAergic neurons throughout the brain. GABA-A agonists quickly alleviate catatonic symptoms in most patients, indicating that dysregulation of this neurotransmitter system is involved in catatonia as well. It was hypothesized that catatonic symptoms in anti-NMDAR-encephalitis may arise from NMDAR hyperfunction causing dysregulation of the GABA-A function (Daniels, 2009; Inta et al., 2015). Besides its role in postsynaptic density, it was found that SHANK3 may function to enrich hyperpolarization-activated cyclic nucleotide-gated (HCN) channels at the postsynaptic sites. The current produced by HCN channels is known as I(h) and plays a key role in controlling rhythmic activity of spontaneously firing neurons (Azene et al., 2003; Biel et al., 2009). Loss of SHANK3 function selectively and severely impairs I(h) currents during neuronal development (Yi et al., 2016). External lithium has a positive effect on HCN gating by producing depolarizing activation shift of these channels (Azene et al., 2003). This may be an explanation for the positive effect of lithium on catatonia-like deterioration previously described in two patients with *SHANK3* deletions (Serret et al., 2015).

Trisomy 21 was diagnosed in one catatonic male, patient 3. This chromosomal disorder has been described in association with catatonia in 7 patients (Ghaziuddin et al., 2015; Jap and Ghaziuddin, 2011; Torr and D'Abrera, 2014). The neurobiological mechanism underlying catatonia in Down syndrome is yet unknown and no candidate genes have been described until now.

The duplications on 16p11.2 and 22q11.2, detected in patient 4 and 5 respectively, are known susceptibility loci for psychiatric pathologies and intellectual disability, but not for catatonia. Although catatonia commonly co-occurs with psychiatric conditions, we cannot deduce unequivocally that CNVs that predispose to psychiatric or intellectual disorders, can also provoke catatonia. Therefore, these duplications were considered likely pathogenic, rather than causal for catatonia in these patients. Awareness of catatonic events in patients carrying similar duplications, as well as systematic CNV studies in other catatonia cohorts, are required to confirm that duplications on 22q11.2 and 16p11.2 can indeed cause catatonia.

The duplications on 2q36.1, 16p13.13 and 17p13.3 were considered variants of unknown significance, since no sufficient data could be retrieved to link these CNVs or the duplicated genes within these regions, to either catatonia or to psychiatric disorders or intellectual disability. Unfortunately, as for most of the patients included in this study, no parental samples could be retrieved to test inheritance. The absence of parental DNA is a common constraint for genetic studies in institutionalized adult patients, and obviously complicates CNV interpretation.

The 14q11.2 duplication in patient 6 has not been linked to catatonia previously. A literature search revealed thirteen additional patients with partially overlapping duplications, sized less than 5 Mb. Intellectual disability or psychiatric/behavioral disorders were present in all patients for which clinical details could be retrieved. The frequent *de novo* occurrence of 14q11.2 duplications (5 out of 8 probands) alludes to a reduced reproductive fitness associated with this genotype. This is in line with the finding that such duplications have not been described in CNV studies in normal controls. All duplication breakpoints were unique, advocating non-homologous end-joining as the underlying mutagenic mechanism. Disregarding the 74kb duplication in DECIPHER patient 256272, the smallest region of overlap comprises only the gene *SUPT16H*, which encodes a subunit of the ‘facilitates chromatin transcription complex’. This

complex acts as a histone chaperone to mediate mRNA elongation, DNA replication and DNA repair (Belotserkovskaya et al., 2003). 14q11.2 deletions, affecting *SUPT16H* and its neighboring gene *CHD8*, are recurrently associated with intellectual disability, autism and macrocephaly (Drabova et al., 2015; Prontera et al., 2014; Terrone et al., 2014; Zahir et al., 2007). Pathogenic variants within *SUPT16H* have not been described, whereas patients with disruptive *CHD8* mutations constitute a subgroup of autism, associated with macrocephaly (80%), gastrointestinal complaints and facial dysmorphism, characterized by pronounced supraorbital ridges, hypertelorism, down-slanting palpebral fissures, a broad nose and pointed chin (Bernier et al., 2014; Neale et al., 2012; O'Roak et al., 2012). Recently, Smyk *et al.* reported similar facial features and relative macrocephaly in a patient with a *de novo* duplication on 14q11.2, comprising both *SUPT16H* and *CHD8* (Smyk et al., 2016). This phenotypic overlap between deletions and duplications on 14q11.2 was not reproduced in this study. Although patients with 14q11.2 duplications presented both a genetic and phenotypic heterogeneity, microcephaly is clearly more common than macrocephaly in this cohort. Other recurrent features included intellectual disability, ranging from mild to severe, epilepsy, hypogonadism, psychiatric disorders (autism) and behavioral problems (hyperactivity, aggression, hyperphagia). The question remains whether these features can all be attributed to the gene *SUPT16H*, or whether copy number gain of other genes within 14q11.2 can modify *SUPT16H* expression or independently cause psychiatric disorders or intellectual disability. A phenotypic effect due to gain of the neighboring gene *CHD8* should be considered, although this gene is only affected in a subset of 14q11.2 duplication patients. *HNRNPC*, proximal to *SUPT16H*, encodes a nuclear ribonucleoprotein involved in (pre-)mRNA processing, and is the only duplicated gene in DECIPHER patient 256272. This 74kb duplication was inherited from a healthy mother. Further genomic studies in psychiatric patients will be required to determine whether gain-of-function mutations in either *SUPT16H*, *CHD8* or *HNRNPC* provoke psychiatric disorders. We conclude that duplications on 14q11.2 predispose to intellectual disability and a broad spectrum of behavioral and psychiatric problems. Apart from patient 6, no other 14q11.2

duplication patients were diagnosed or reported with catatonia. Therefore, in accordance with our CNV interpretation algorithm, this 14q11.2 duplication was considered causal for intellectual disability and likely pathogenic for catatonia in this patient.

The identification of catatonia-related copy number changes in this study, underscores the importance of genetic research in patients with catatonia, in order to untangle the neurobiological background, and to uncover novel therapeutic targets for this disorder.

Studying psychiatric disorders in intellectually disabled adults is challenging, since an accurate psychiatric diagnosis becomes difficult as the level of intellectual functioning declines. The applicability of existing standardized classification systems such as DMS for persons with intellectual disability is debatable (Fletcher et al., 2009). Although most catatonic signs are non-verbal and can be clinically detected, the severity of some symptoms like agitation, mutism and grimacing may be different from catatonic patients without intellectual disability. Future studies are required to validate clinical criteria for catatonia in patients with intellectual disability.

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WEB RESOURCES

DECIPHER – <https://decipher.sanger.ac.uk/>

The database of genomic variants – <http://dgv.tcag.ca/dgv/app/home>

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FIGURE LEGENDS

Figure 1. Molecular overview of 14q11.2 duplications, identified in patient 6 and in additional patients retrieved from literature or DECIPHER. Blue bars depict the duplicated regions in well-phenotyped patients. Grey bars present duplications in patients from whom limited (DECIPHER 284790, 287656 and 258497) or no clinical data (DECIPHER 322324 and 292187) could be retrieved. The red box delineates the minimal region of overlap, excluding the duplication in DECIPHER patient 256272. Abbreviations: dn: *de*

novo, mat: maternally inherited, ?: unknown inheritance, ID: intellectual disability, Ψ : psychiatric or behavioral disorder.

SUPPLEMENTARY DATA

Supplementary figure 1. CNV interpretation algorithm for catatonia. (1) A CNV was considered causal, either when this CNV is completely overlapping with chromosomal syndromes, known to cause catatonia, or when this CNV is harboring catatonia-related genes. (2) CNVs were considered likely pathogenic, when this CNV lays within the critical region of a chromosomal syndrome. (3) CNVs smaller than 200kb in size and not fulfilling the criteria under (1) or (2) were discarded from further analysis. (4) CNVs known to be prevalent in more than 1% of the normal population were considered benign. (5) All remaining rare CNVs were considered variants of unknown significance, and were further examined, based on gene content, inheritance and phenotype correlations with similar chromosomal variants.